STIC-ILL

2/24

From: Afremova, Vera 1651
Sent: Monday, February 24, 2003 1:54 PM
To: STIC-ILL
Subject: 10/089,120

433076

Hi, please, could I have this ref.:

Hasegawa, T. 1988. Actinomycetologica 2:31-45.

8813973

Vera Afremova
CM1 11E13
308-9351

9794560 DL-NE

~~9716095~~ = NO

-NO JICST 3/5

ACTINOMYCETOLOGICA Vol. 2 No. 1, 31–45, 1988

Actinokineospora: A New Genus of the *Actinomycetales*

TŌRU HASEGAWA

Institute for Fermentation, Osaka

17-85, Juso-honmachi 2-chome, Yodogawa-ku, Osaka 532, Japan

(Received for publication March 3, 1988)

A new genus *Actinokineospora* is described. It is characterized by forming chains of zoospores originating from aerial mycelium, and has type IV/A cell walls and a type PII phospholipid pattern. The major menaquinone is MK-10. No mycolic acids are present. The guanine-plus-cytosine content of the deoxyribonucleic acid is 72.0 mol%. The type strain of *A. riparia* is C-39162 (IFO 14541).

In the course of a screening program to detect antibiotic producers among rarely occurring members of genera of the order *Actinomycetales*, an organism was isolated that had morphological and chemotaxonomic characteristics that precluded its placement in any of the previously described genera. This organism is described here. The isolated motile organism cannot be accommodated in any of the previously described genera of the actinomycetes, since the organism has type IV/A cell walls. I propose to include it in a new genus, *Actinokineospora*, the type species of which is *A. riparia*.

MATERIALS AND METHODS

Bacterial strains

Strain C-39162^T was isolated from a soil sample collected at the side of the Ado River, Shiga Prefecture, Japan.

Morphological characterization.

The morphology of the aerial mycelia grown on sucrose nitrate agar[1] at 28°C for 14 days was studied by light microscopy. A cover slip culture of yeast extract-malt extract agar (ISP medium 2) used for light microscopy was prepared by the method of Kawato and Shinobu[2]. For scanning electron microscopy, an agar block containing numerous spores on sucrose nitrate agar was gradually dehydrated with increasing amounts of ethanol and finally dried by the critical point method[3]. Each specimen was coated with evaporated gold and examined with a Hitachi model

S-570 scanning electron microscope. For transmission electron microscopy, the agar blocks containing colonies were in Kellenberger buffer (pH 6.5) containing 1% osmium tetraoxide and stored at 4°C for 18 h. The agar blocks were washed twice with Kellenberger buffer (pH 6.5) containing 0.5% uranyl acetate for 2 h at room temperature. They were dehydrated through a series of ethanol, and propylene oxide. They were finally embedded in Epon 812 (Ladd Res. Labs., Burlington, Vermont, U.S.A.) and polymerized at 35°C for 24 h, 45°C for 24 h, and 62°C for 24 h. Then sections were cut with a diamond knife on a LKB microtome model Ultratome III. The sections were stained with lead citrate. The prepared specimens were examined with a JEOL electron microscope model JEM-1200EX. The zoospores from aerial mycelia were observed by the method of Hasegawa et al.[4] To observe the flagella of the zoospores, one drop of a suspension containing them was placed on Formvar coated 300-mesh copper grids, and air dried. The specimens were shadowed with platinum-paladium and examined with a JEOL electron microscope model JEM 100B.

Culture and physiological characterization.

For culture and physiological characterization, the media recommended by Waksman[1] and endorsed by ISP[5] were used. The cultures were incubated at 28°C and observed for 14 days. The colors were compared with that in the Color Harmony Manual.[6] Carbohydrate utilization was determined by the method of Pridham and Gottlieb.[7] Temperature requirements and the effect of pH for growth were those employed by Gordon and Mihm,[8] and Gordon.[9]

Susceptibility to antibiotics and antimicrobial agents.

The susceptibility to drugs was determined by placing susceptibility disks (Showa Disk Co., Ltd.) on ISP medium 2 which had been seeded with a suspension of strain C-39162^T. After incubation for 7 days at 28°C, the diameters of the inhibition zone were measured.

Chemotaxonomy.

Whole-cell hydrolysates and purified cell wall hydrolysates were analyzed by the methods of Hasegawa et al.[10], and Becker et al.,[11] respectively. Fatty acids and phospholipids analyses were carried out using the procedures of Lechevalier et al.[12] Mycolic acids were determined by the method of Minnikin et al.[13] The procedures used for gram staining and acid-fast staining were those described in the Manual of Clinical Microbiology, 2nd ed.[14]

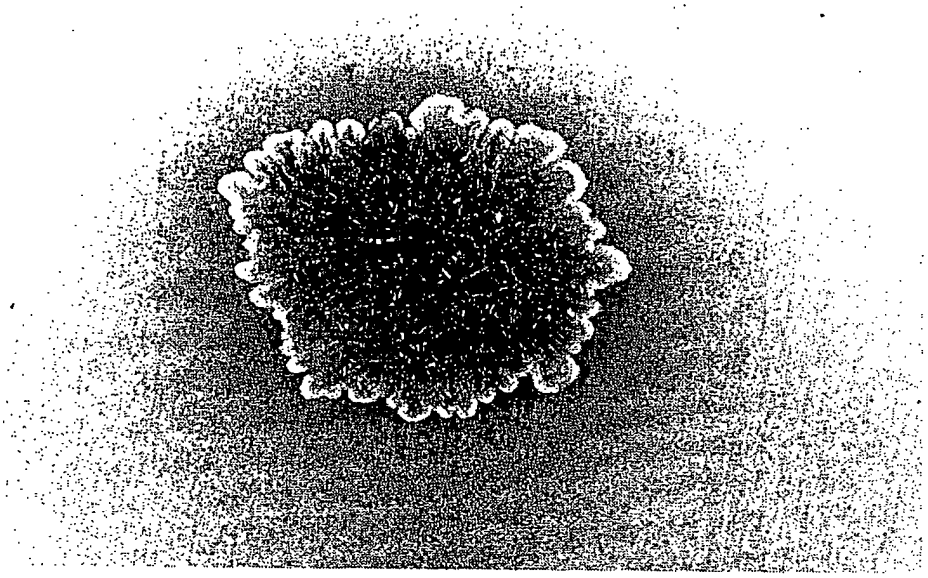

Fig. 1. A giant colony on yeast extract-malt extract agar.

Fig. 2. Micrograph of thin growing colonies bearing white aerial mycelia on sucrose nitrate agar.

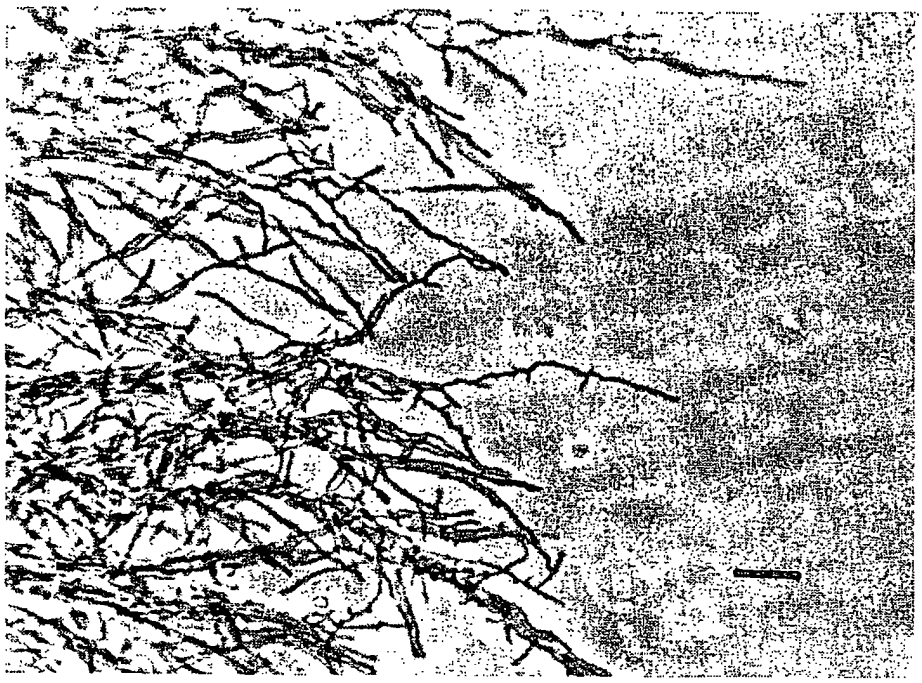

Fig. 3. Micrograph of vegetative mycelia in a cover slip culture of yeast extract-malt extract agar. Bar=20 μm.

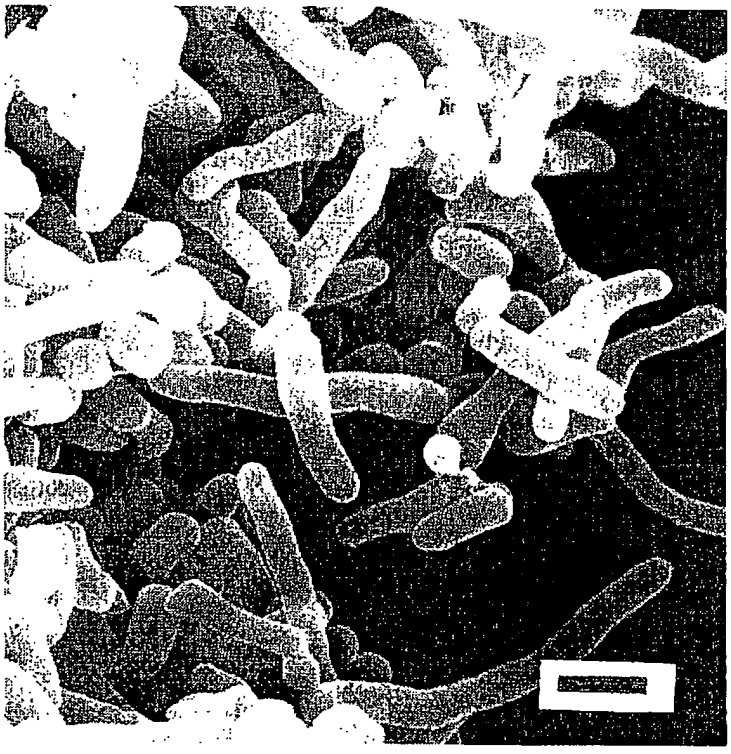

Fig. 4. Scanning electron micrograph of fragmented cells in tryptone yeast extract broth by shaking culture. Bar=1 μm.

Fig. 5. Micrograph of the aerial mycelium on sucrose nitrate agar. Bar=20 μm.

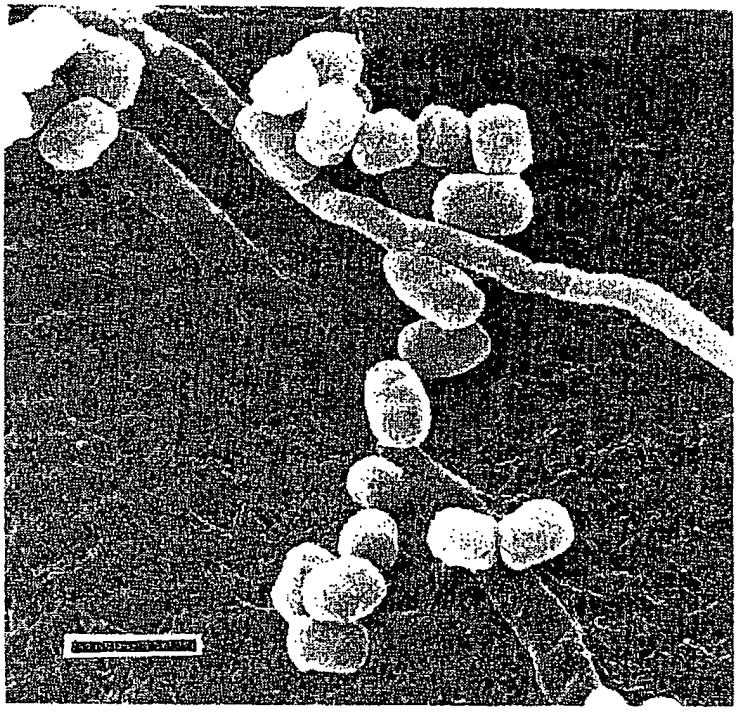

Fig. 6. Scanning electron micrograph of irregular spore chains of the aerial mycelium. Bar=1 μm.

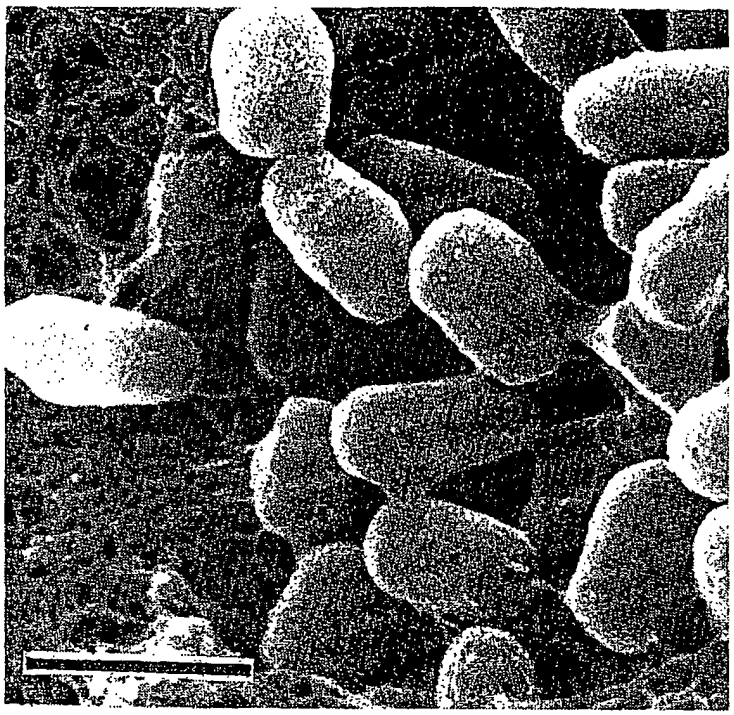

Fig. 7. Scanning electron micrograph of irregular spore chains appearing from the agar medium. Bar=1 μm.

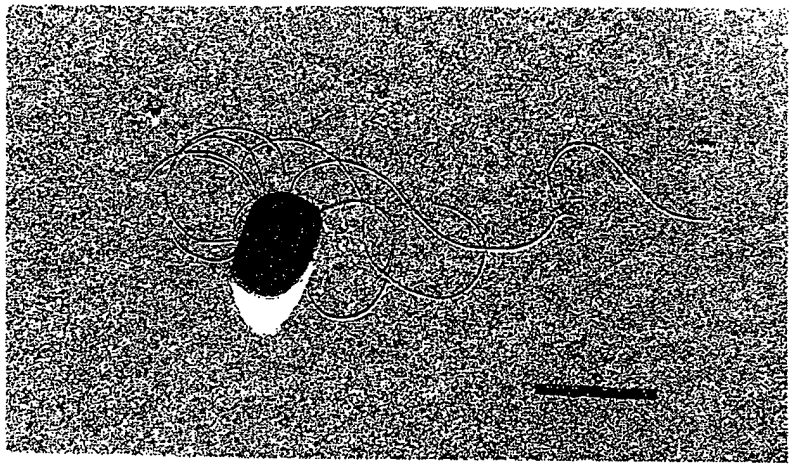

Fig. 8. Electron micrograph of a zoospore. Bar=1 μm.

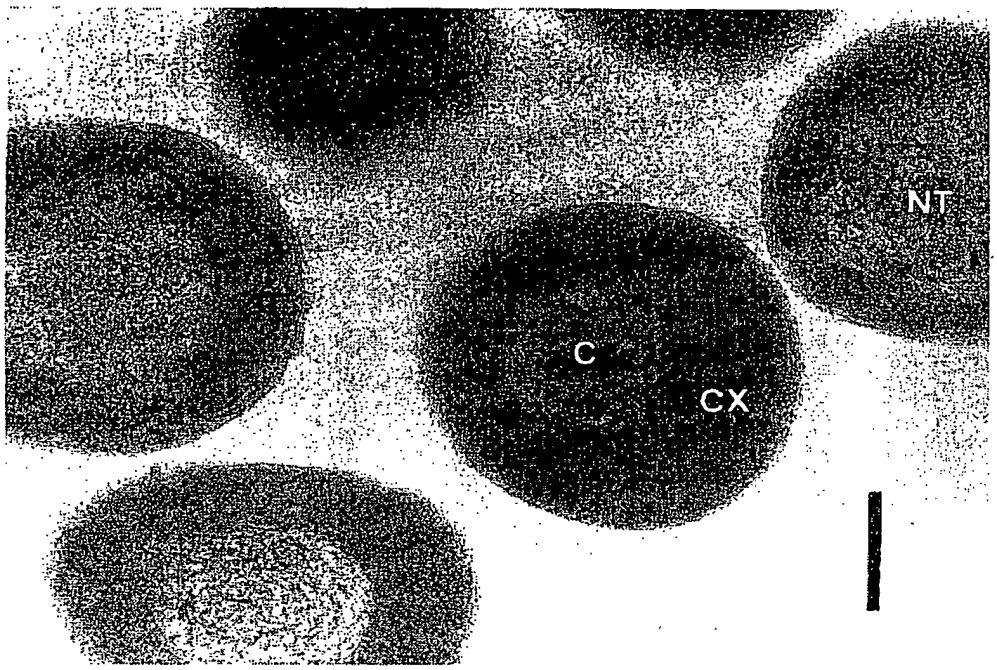

Fig. 9. Electron micrograph of a thin section of spores. Bar=200 μm
C, core region; CX, cortex-like coat; NT, net-like texture.

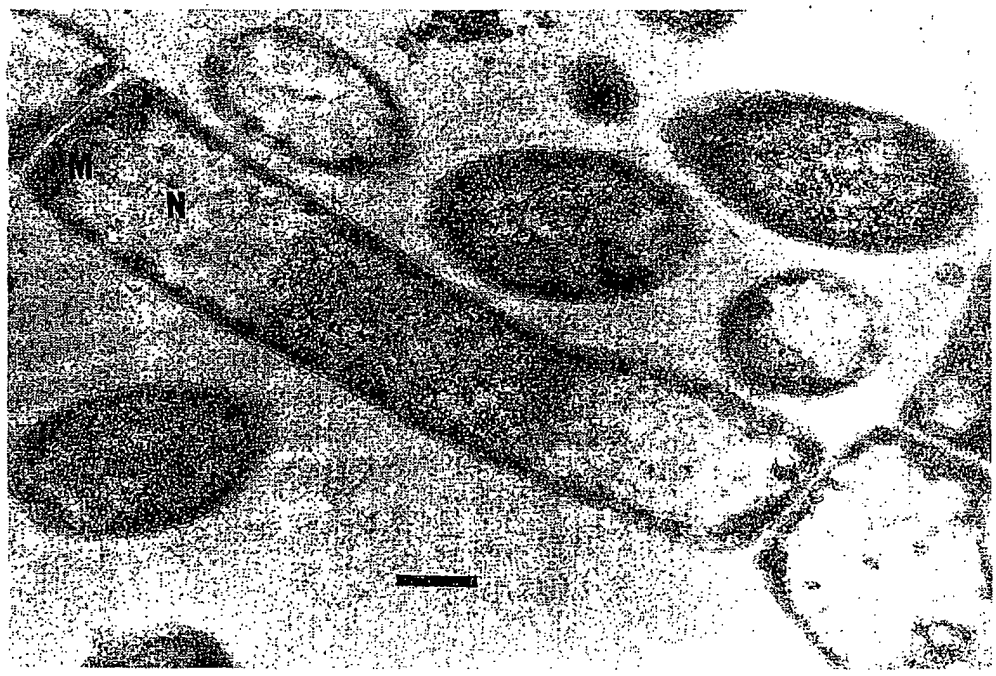

Fig. 10. Electron micrograph of a longitudinal section of the vegetative mycelium. Bar=200μm.
N, nuclear region; M, lamellar mesosome.

(Fig. 8). After 24 h of incubation, several germ tubes are formed and motility ceases.

The structural features of a matured spore of strain C-39162^T are shown in Fig. 9. The spore is covered with multilayered integuments and its core is separated from the cortex-like coat by a boundary region. A net-like texture is visible in the spore core. A longitudinal section of a vegetative mycelium shows lamellar mesosomes associated with septum formation and a nuclear region (Fig. 10).

Culture characteristics.

The culture characteristics of strain C-39162^T on various media are shown in Table 1. On organic or chemically defined media, in general, the vegetative mycelium is colorless to brownish, and white aerial mycelium is formed, but not abundantly. A soluble pigment is not produced except on ISP medium 2, on which a pale brown pigment is produced. No melanoid pigments are produced on ISP media 1, 6 and 7.

TABLE 1. Cultural characteristics of strain C-39162^T

Sucrose nitrate agar
- G (Growth): poor, thin, colorless
- A (Aerial mycelium): poor or moderate, powdery, white
- R (Reverse side color): colorless
- S (Soluble pigment): none

Glucose asparagine agar
- G: poor or moderate, Light ivory (2ca) to Honey gold (2ic) to Mustard brown (2pi)
- A: none
- R: colorless to 2ca to 2pi
- S: none

Glycerol asparagine agar
- G: moderate, colorless
- A: none
- R: colorless
- S: none

Calcium malate agar
- G: none or very poor
- A: none or poor, white
- R: colorless
- S: none

Water agar
G: very poor, thin, colorless
A: none
R: colorless
S: none

Tyrosine agar
G: poor, thin, colorless
A: none
R: colorless
S: none

Inorganic salts starch agar
G: poor, colorless to Light ivory (2ca)
A: Poor, white
R: colorless
S: none

Yeast extract malt extract agar
G: moderate, colorless to Cork tan (4ie) to Chestnut brown (4ni)
A: none or poor, white
R: colorless
S: pale brown

Nutrient agar
G: poor or moderate, Light ivory (2ca)
A: none
R: colorless
S: none

Oatmeal agar
G: poor or moderate, colorless
A: none
R: colorless
S: none

Bennett's agar
G: moderate, colorless to 4ie to 4ni
A: poor, white
R: colorless to 4ie to 4ni
S: pale brown

Potato carrot agar
G: poor, thin, colorless
A: poor, powdery, white
R: colorless
S: none

Peptone yeast extract iron agar
G: poor or moderate, Light ivory (2ca)
A: none
R: colorless
S: none

Physiological characteristics.

The physiological characteristics of strain C-39162^T are summarized as follows. The strain does not grow under anaerobic condition when the GasPak system (BBL Microbiology Systems) is used. Growth takes place between 23 and 41°C and at between pH 5.0 and 9.0. No growth occurres in lysozyme broth or on medium supplemented with 5% NaCl. The proportion of visible spores is reduced to less than 1 in 10,000 by heating in a boiling water bath for 5 min. Gelatin is weakly liquefied. Milk is not peptonized and not coagulated; starch is not hydrolyzed. Tyrosine is decomposed, but neither xanthine, hypoxanthine, nor adenine are decomposed. D-glucose, trehalose, and soluble starch are utilized as carbon sources for growth. Little or doubtful growth is obtained with cellulose, D-fructose, melibiose, esculin, mannose, sucrose, inulin, and D-sorbitol; no growth is obtained with adonitol, D-mannitol, L-arabinose, rhamnose, glycerol, erithritol, sulcitol, *i*-inositol, D-xylose, D-galactose, maltose, lactose, raffinose, salicin, ribose, and L-sorbose.

Susceptibility to antibiotics and an antimicrobial agent.

Strain C-39162^T is resistant to ampicillin (30 μg), kanamycin (50 μg), and nalidixic acid (50 μg). Tetracycline (200 μg) produces 24-mm zones; erythromycin (50 μg) produces 65-mm zones; chloramphenicol (100 μg) produces 35-mm zones; lincomycin (30 μg) produces 34-mm zones; novobiocin (20 μg) produces 51-mm zones; gentamicin (30 μg) produces 25-mm zones; dihydrostreptomycin (50 μg) produces 35-mm zones; and polymyxin B (100 U) produces 12-mm ones.

Antibiotic properties.

Strain C-39162^T produces a macromolecular compound with anti-mycoplasmal activity.

Chemotaxonomy.

Whole-cell hydrolysates contained galactose, glucose, mannose, arabinose, and rhamnose. Purified cell wall preparations contain *meso*-diaminopimelic acid, glutamic acid, alanine, a trace of glycine, muramic acid, glucosamine, galactose, glucose, and a minor amount of arabinose. Molar ratios of galactose and arabinose in the hydrolysates of whole-cell or cell wall are shown in Table 2. The phospholipids present are phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylinositol. Thus, strain C-39162^T has type IV cell walls, a type A sugar pattern, and a type PII phospholipid composition. The lipids contain mostly branched-chain fatty acids (data not shown). Mycolic acids are not present. The menaquinones present are MK-10, and a minor amount of MK-9 and MK-11.

Young mycelia grown under submerged conditions are gram positive and not acid fast.

The guanine-plus-cytosine of DNA extracted from strain C-39162^T is 72.0 mol%.

Table 2. Molar ratios of galactose and arabinose in the hydrolysates of whole-cell or cell wall

	Component	Molar ratio
Whole-cell	Galactose	1.00
	Arabinose	0.51
Cell wall	Galactose	1.00
	Arabinose	0.05

Type strain.

Strain C-39162^T, the type strain of *Actinokineospora riparia*, has been deposited in the Institute for Fermentation, Osaka, as strain IFO 14541. Because the species description given above is based on a single strain, it serves as the description of the type strain.

Aerobic actinomycetes known to form motile elements[15] are found in the halocarpic genera *Dermatophilus* and *Geodermatophilus*; in the gram positive to gram variable genus *Oerskovia*; in seven sporangium forming members of *Actinoplanaceae*; in the LL-diaminop diaminopimelic acid containing genera *Kineosporia* and *Sporichthya*; and in the synnema forming genus *Actinosynnema*. Among those genera, only *Sporichthya* and *Actinosynnema* are characterized by the

formation of chains of zoospores originating from aerial mycelium. *Actinokineospora* develops vegetative mycelium well in agar medium. It does not form synnema originating from the vegetative mycelium. Thus, *Sporichthya* and *Actinosynnema* are readily differentiated from *Actinokineospora*.

From a chemotaxonomic point of view, most motile actinomycete genera have type II or III cell walls except for *Kineosporia* (type I), *Sporichthya* (type I) and *Oerskovia* (type VI). Therefore, no motile actinomycetes that have type IV cell walls are currently known. A comparison of *Actinokineospora* with the relevant genera is shown in Table 3.

Table 3. Differentiation of the genus *Actinokineospora* from known genera of motile actinomycetes

	Aerial mycelium formation	Sporangium formation	Cell wall type	Whole-cell sugar pattern
Kineosporia	-	+	I	A/D
Sporichthya	+	-	I	NC
Actinoplanes	-	+	II	D
Ampullariella	-	+	II	D
Dactylosporangium	-	+	II	D
Pilimelia	-	+	II	D
Actinosynnema	+	-	III	C
Dermatophilus	-	-	III	B
Geodermatophilus	-	-	III	C
Planomonospora	+	+	III	B
Planobispora	+	+	III	B
Spirillospora	+	+	III	B
Streptoalloteichus	+	+	III	C
Actinokineospora	+	-	IV	A
Oerskovia	-	-	VI	C

Actinokineospora is not a member of the *Nocardiaceae*, which has type IV/A cell walls, because the pure cell wall preparations of this organism contain large amounts of galactose and glucose, and only trace amounts of arabinose as above-mentioned. Because, the bound form of the arabinose in the cell wall is different from the members of *Nocardiaceae* with major amounts of arabinose and galactose. The bound form of the arabinose in the cell wall may resemble that of the non-motile actinomycete genus *Kibdelosporangium*.[16]

Pending the isolation of more strains of actinomycetes forming aerial mycelia with chains of zoospores, the genus *Actinokineospora* should be considered one of the genus of the order *Actinomycetales* which are of uncertain familial placement.

ACKNOWLEDGEMENTS

I thank Dr. H. Kuraishi, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, for the menaquinone analysis. I also thank Drs. Y. Sugino, Y. Nakao, H. Okazaki and T. Iijima, for their encouragement. I am indebted to Dr. A. Yokota for the sugar analysis by HPLC, Dr. M. Nomura for advice on transmission electron microscopy, and Dr. H. Ono for determining the DNA base composition. The technical assistance of Messrs. Y. Kōno and H. Tatekawa is greatfully acknowledged.

LITERATURE CITED

1) Waksman, S.A.: The Actinomycetes vol. 2. The Williams and Wilkins Co., Baltimore, 1961
2) Kawato, M. and R. Shinobu: On *Streptomyces herbaricolor* nov. sp. Supplement: a simple technique for the microscopical observation. Mem. Osaka Univ. Lib. Arts Educ. 8: 114-119, 1959
3) Anderson, T.F.: Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Trans. N.Y. Acad Sci. Ser. 2, 13: 130-134, 1951
4) Hasegawa, T.; S. Tanida, K. Hatano, E. Higashide and M. Yoneda. Motile actinomycetes: *Actinosynnema pretiosum* subsp. *pretiosum* sp. nov., subsp. nov., and *Actinosynnema pretiosum* subsp. *auranticum* subsp. nov. Int. J. Syst. Bacteriol. 33: 314-320, 1983
5) Shirling, E.B. and D. Gottlieb: Method for classification of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313-340, 1966
6) Jacobson, F.; W.C. Granville and C.E. Fogs: Color harmony manual. 4th ed. Container Corporation of America, Chicago, 1958
7) Pridham, T.G. and D. Gottlieb: The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. J. Bacteriol. 56: 107-114, 1948
8) Gordon, R.E., and J.M. Mihm: Identification of *Nocardia caviae* (Erikson) nov. comb. Ann. N.Y. Acad. Sci. 98: 628-636, 1962
9) Gordon, R.E.; D.A. Barnett, J.E. Handerhan and C. HorNay Pang: *Nocardia coeliaca, Nocardia autotrophica*, and the nocardin strain. Int. J. Syst. Bacteriol. 24: 54-63, 1974
10) Hasegawa. T.; M. Takizawa, and S. Tanida: A rapid analysis for chemical grouping of aerobic actinomycetes. J. Gen. Appl. Microbiol. 29: 319-322, 1983

11) Becker, B.; M.P. Lechevalier and H.A. Lechevalier: Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl. Microbiol. 13: 236-243, 1965
12) Lechevalier, M.P. and H.A. Lechevalier: The chemotaxonomy of actinomycetes. *In* "Actinomycete taxonomy, ed. by A. Dietz and D.W. Thayer", pp. 227-291, Society for Industrial Microbiology, 1980
13) Minnikin, D.E.; L. Alshamaony and M. Goodfellow: Differentiation of *Mycobacterium, Nocardia,* and related taxa by thin layer chromatographic analysis of whole-organism methanolysates. J. Gen. Microbiol. 88: 200-204, 1975
14) Paik, G. and M.T. Suggs: Reagents, stains and miscellaneous test procedures. *In* "Manual of Clinical Microbiology, 2nd ed. by E.H. Lanette, E.H. Spaulding and J.P. Truant", pp. 930-950, American Society for Microbiology, 1974
15) Lechevalier, H.A. and M.P. Lechevalier: Introduction to the order *Actinomycetales.* In "The prokaryotes: a handbook on habitats, isolation and identification of bacteria, ed. by M.P. Starr, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel", pp. 1915-1922, Springer-Verlag, Berlin, 1981
16) Shearer, M.C.; P.M. Colman, R.M. Ferrin, L.J. Nisbet and C.H. Nash III: New genus of the *Actinomycetales: Kibdelosporangium aridum* gen. nov., sp. nov. Inst. J. Syst. Bacteriol. 36: 47-54, 1986